EVALUATION OF DIAZO TEST IN THE EARLY DIAGNOSIS OF TYPHOID FEVER

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DOCTOR OF MEDICINE
(PEDIATRICS)

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C E R T I F I C A T E

This is to certify that the work entitled "EVALUATION OF DIAZO TEST IN THE DIAGNOSIS OF TYPHOID FEVER", has been carried out by Dr. Yuganter Pandey in the department of Paediatrics, M.L.B. Medical College, Jhansi.

He has put in the necessary stay in the department as per university regulations.

Dated : 17.11.95

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C E R T I F I C A T E

This is to certify that the work entitled "EVALUATION OF DIAZO TEST IN THE DIAGNOSIS OF TYPHOID FEVER", which is being submitted as a thesis for M.D. Pediatrics) Examination, 1996, of BundelKhand University, has been carried out by Dr. Yuganter Pandey under my direct supervision and guidance. The techniques embodied in the thesis have been undertaken by the candidate himself and the observations recorded have periodically been checked and verified by me.

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C E R T I F I C A T E

This is to certify that the work entitled "EVALUATION OF DIAZO TEST IN THE DIAGNOSIS OF TYPHOID FEVER", has been carried out by Dr. Yuganter Pandey under my guidance and supervision. The observations recorded have been checked and verified by me from time to time. The techniques embodied in this thesis have been embodied by the candidate himself.

Dated : 16/11/95

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Dated : 16/11/95

(Yugantar Pandey)

CONTENT

I N T R O D U C T I O N

INTRODUCTION

Typhoid - the name comes from Greek work "typhos" meaning "stupor". Headache, mounting fever and mental dullness precedes stupor as S. typhi or S. paratyphi disseminate in blood and reticuloendothelial system. Term 'Enteric fever' or 'Typhus abdominalis' covers infection caused by S. typhi or S. paratyphi A, B or C.

Typhoid fever is unique to humans, world wide in distribution and occurs irrespective of climate. Failure in personal hygiene, particularly in public healthy precautions, commonly result in appearance of typhoid fever even in well sanitated countries. Nevertheless with improved water supplies and sanitation in the better developed temperate climate, its incidence has steadily decreased and it is now much more prevalent in the tropics than in colder parts of the globe. Typhoid fever continues to be unabated in developing countries of Africa, Asia and Latin America where sanitation and water supplies are still substandard.

In these countries incidence rate varying from 100 to 1500 per 100,000 population has been reported by different workers, while in developed countries like United States of America, where 36000 cases were detected in year 1920, the incidence has dropped to 500 cases per year (Keusch, 1994).

In India typhoid fever is a major health problem. It continues to exist as an endemic disease due to poor sanitation and low socio-economic status.

Typhoid fever is caused by S. typhi, the most important member of genus Salmonella.

Typhoid bacillus was first observed by Eberth in the year 1880 in mesenteric nodes and spleen of fatal cases of typhoid fever and was isolated by Gaffky (1884). It came to be known as Eberth Gaffky bacillus or Eberthella typhi (Ananthanarayan and Paniker, Text book of Microbiology, 3rd edn.).

Typhoid fever can be diagnosed by blood culture, stool culture, urine culture and widal test.

Blood culture is the only test available that can be done in the first week of illness but the results are obtained quite late and it has its own limitations.

Other tests can be done only after 1 week of illness and are time consuming and less sensitive.

Diazo test is a simple bed side test which can give early clue to the diagnosis of typhoid fever but very little work has been done to evaluate this and it needs further attention for its role in early and diagnosis of typhoid fever.

Huckstep (1962) was the first worker to have evaluated the efficacy of diazo test, a simple bedside urine test in the early diagnosis of typhoid fever. Very few workers since then have evaluated this test for diagnosing typhoid fever, to test its specificity and sensitivity viz-a-viz blood culture and widal test which are the conventional tests to diagnose typhoid fever.

In the light of these observations made by Huckstep, the present venture was directed to evaluate the diazo test in early diagnosis of typhoid fever.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Typhoid fever is a distinctive acute systemic febrile infection of mono-nuclear phagocytes. Man is the only known reservoir. Clinical typhoid fever is almost always a human adapted salmonella and most of the cases can be traced to a human carrier. Food handlers and cooks who become carriers are particularly dangerous. The best known of such typhoid carriers was Mary Mallen (Typhoid Marry), a New York cook who, over a period of 15 years caused at least 7 outbreaks affecting over 200 persons.

Typhoid fever was once prevalent all over the world and was not well differentiated from other prolonged fevers.

A detailed study of the disease was presented by Bretonneau (1820) who identified the intestinal lesions. The name 'typhoid' was given by Louis (1829) to distinguish it from typhus fever.

Budd (1856) pointed out that the disease was transmitted through the excreta of patients. Eberth

(1880) described the typhoid bacillus and Gaffky(1884) isolated it in pure culture. Its causative role was confirmed by Metchnicoff and Besredka (1900) by infecting apes experimentally. Subsequently S. paratyphi A was isolated by Gwyn (1898), S. paratyphi B (S. schott muelleri) by Achard and Bensaude (1896) and S. paratyphi C (S. birschfeldii) by Uhlenhuth and Hubener (1908) from cases resembling typhoid fever.

MAGNITUDE OF PROBLEM

Typhoid fever has been virtually eliminated from the advanced countries during the last several decades mainly as a result of improvement in water supply and sanitation but it continues to be endemic in poor nations of the worlds. The control of paratyphoid fever has not been so successful.

With improvement in environmental sanitation in U.S.A., the incidence of typhoid fever has gradually dropped. Data on incidence of typhoid fever has revealed that while in 1920 almost 36000 cases were detected, the annual number of cases has drastically

dropped to approximately 500 by the year 1966(Harrison's Principles of Internal Medicine, 13th ed.). The incidence in U.S.A. has dropped five folds from 1955 to 1966(from 1 per 100000 population to 0.2 per 100000 population) and has remained steady since then (Harrison' Principles of Internal Medicine, 13th edn.). Now most of the cases that occur are either acquired abroad or imported by immigrants.

In U.K. typhoid fever has been brought very close to eradication with approximately 1 case per 1000000 population (Park's Text book of Social & Preventive Medicine, 13th edition).

Typhoid fever continues to be unabated in the developing countries of Africa, Asia and Latin America. In these countries incidence rates varying from 100 to 1500 per 100,000 population have been reported (Park and Park, 1995).

PROBLEM IN INDIA

Typhoid fever is endemic in all parts of India. Health surveys conducted by the Central Ministry

of Health in community development areas, indicated a morbidity rate varying from 102 to 2219 per 100,000 population in different parts of country. Patnaik (1967) reported an incidence of 110 among males and 75 among females per 10,000 population in Delhi. A limited study in an urban slum showed 1 percent of children upto 17 years of age suffer from typhoid fever every year (Park and Park, 1995). Statistics for the period of 1980 to 1986 showed an average more than 300,000 cases of typhoid fever each year (Kumar et al, 1988). In the absence of reliable epidemiological data these figures do not reflect the true incidence of typhoid in India.

In the recent past, most centres in India have experienced a sudden spurt in the incidence of typhoid fever and recently in India a new phenomenon has been reported with typhoid fever. There have been out breaks of chloramphenicol resistant typhoid cases. This started in 1972 when there was a out break of chloramphenicol resistant S. typhi in Mexico. A similar out break occurred around the same time in North Kerala.

Subsequently the Salmonella phage typing centre, Lady Hardinge Medical College, New Delhi isolated multiple drug resistant strains from outbreaks in several parts of India including Bombay and Chandigarh. This problem was not very evident in 1980s. Again in 1990s, virulent typhoid infection resistant to chloramphenicol has encountered in Western India.

The proportion of typhoid to paratyphoid A is about 10 : 1. Paratyphoid B is rare in India but common in East Europe. Paratyphoid C is still rare (Ananthnarayan and Paniker, 1989).

AGENT FACTOR

Salmonella are gram negative rods, motile with peritrichate flagella. They do not form spores or capsule.

Salmonellae are aerobic and facultative anaerobes, grow readily on simple media, colonies are large, 2-3 mm in diameter, circular, low convex and smooth.

On McConkey agar and deoxycholate citrate medium colonies are colourless due to absence of lactose fermentation.

On Wilson Blair bismuth sulphate medium jet black colonies with a metallic sheen are formed due to production of H_2S.

ANTIGENIC STRUCTURE

Salmonella possess the following antigens -

1. Flagellar antigen - H
2. Somatic antigen - O
3. Surface antigen - Vi

'H' antigen is heat labile protein and forms large loose, fluffy clumps when mixed with antisera. 'O' antigen is phospholipid protein polysaccharide complex and heat stable. It forms chalky granular, compact clumps with antisera.

Vi antigen is related to virulence. Total absence of Vi antigen indicates poor prognosis and its persistence indicates development of carrier state.

MODE OF INFECTION

It occurs in two epidemiological forms. First is endemic or residual typhoid and other is epidemic form. Typhoid epidemics are water borne or milk or food borne and the usual mode is faeco oral.

PATHOGENESIS

Following ingestion of a suitable inoculum, S. typhi pass the gastric barrier to reach the small bowel. It invades the upper small bowel and produces a transient asymptomatic bacteraemia. Organisms are ingested by mononuclear phagocytes and must survive and multiply intracellularly to cause illness.

When number of intracellular bacteria surpasses a critical threshold, secondary bacteraemia occurs and result in invasion of gall bladder and Peyer's Patches of intestine. Sustained bacteraemia is responsible for persistent fever of clinical typhoid while inflammatory responses to tissue invasion determine the pattern of clinical expression.

With invasion of gall bladder and Peyer's Patches, bacteria regain entry to bowel lumen and may be recovered

in stool culture beginning in second week of clinical disease. Seeding of kidney leads to positive urine culture.

CLINICAL MANIFESTATIONS

Symptomatology

Wilcocks and Manson Bahr (1982) reported that usual incubation period for all the enteric infections is about 14 days, but may be shorter than 7 days, or longer than 21 days. There is a wide range in severity of infection from the mildest to the most severe cases.

In the first week of illness, there is high grade continuous fever which mounts to high grade in a step ladder pattern in a typical case(Nelson's text book of Pediatrics, 14th edn.). The pulse rate is 20-40 beats/ min, slower than the probable pulse rate for such height of fever (Manson Bahr and Wilcock, 1982). This relative bradycardia, a characteristic feature of a typical case of typhoid fever, is found only in adult and is not found in paediatric age group (Forfar and Arneil's Text book of Pediatrics, 4th edn.). There is increase in respiratory rate with signs of catarrhal bronchitis. The abdomen becomes distended, there may be either diarrhoea or

constipation initially but by the end of week, there is usually diarrhoea causing 'peak soup' stools (Adams and Maigraith, 1992). The patient becomes drawn and pallid, there is circumscribed flush on the cheek, tongue is furred. General appearance is one of dullness and toxaemia. Typical rash appears on 7th day, restricted to abdomen and flanks, as a rule (Rose Spots), and are sparse (Adams and Maigraith, 1992).

In the second week of illness, temperature is maintained at high level. Toxaemia becomes more marked, thereafter is low muttering delirium. Deafness is usual (Harrison's Principles of Int Med, 13th edn.). There is commonly basal congestion of lungs, spleen becomes palpable and tender and it is soft (Edward and Bouchier, 1995). There is increase in abdominal discomfort.

As disease progresses in third week the symptoms begin to lessen but the grave and common complications, i.e. haemorrhage and perforation, are prone to occur as sloughs separate from ulcers (Adams and Maegraith, 1992).

In favourable cases convalescence is entered upon in fourth week.

RELAPSES

Relapses occur in about 10% untreated cases (Adams and Maegraith, 1992). Ramli (1950) found that relapse rate is 4%, in cases taking antibiotics from 12 days after defervescence of fever, while it was 50% in cases taking antibiotics for shorter periods. Relapses occur usually within 5 days to 10 days of primary attack and are seldom more severe than original attack(Marmion, 1952).

COMPLICATIONS

As there are stages of bacteraemia, in a unfavourable case there are complications involving many systems. These complications include hepatitis, meningitis, nephritis, myocarditis, bronchitis, pneumonia, arthritis, osteomyelitis, parotitis, and orchitis.

Excepting relapse, the frequency of all the complications including haemorrhage and perforation of bowel is reduced by prompt use of antibiotics (Harrison's Principles of Internal Medicine, 13th edn.).

LABORATORY FINDINGS

1. Blood counts and Haemoglobin

In most of cases W.B.C. count is normal but in 25% cases there is leucopenia and neutropenia (Harrison's Text book of Internal Medicine, 13th edn.) In the event of complications there may be secondary leucocytosis.

Anaemia of blood loss may be superimposed on anaemia of the infection.

2. BLOOD CULTURE

In the early stages of illness blood culture is the most conclusive diagnostic method, and it is the only test to diagnose typhoid fever in first week of illness and is positive in 90% cases(Harrison's Principles of Int. Med. 13th edn. and Ananthnarayan & Paniker, 1989).

It is positive in 75% cases in II week, in 60% cases in III week and in 25% cases thereafter. But the biggest disadvantage is that it becomes negative on administration of antibiotics(Ananthnarayan & Paniker,1991).

3. Faecal Culture

S. typhi and even S. paratyphi can be isolated from the faeces throughout the illness, but most frequently it is isolated during II and III week.

According to Ananthnarayan and Paniker (1991) as Salmonellae are shed in faeces throughout the course of illness, it is almost as valuable as blood culture and particularly valuable in patients on antibiotics since drugs do not eliminate bacilli from gut as early as it does from blood.

It is done on brilliant green enrichment medium, tetrathionate broth and Wilson Blair agar medium. Stool should be transported in meat broth.

4. Urine Culture

Salmonellae are shed in urine irregularly and infrequently, hence it is less useful than blood culture.

Generally positive only in II and III week and that too in only 25% of cases(Ananthnarayan & Paniker,1989).

5. Clot Culture

Thomas et al (1954) suggested that this method can diagnose typhoid fever in early stages of disease. Positive results can be obtained in 24 hour by culturing clots free from patients. Serum in streptokinase broth put up in 100 ml bottles containing slopes of Wilson and Blair's medium. Streptokinase broth is prepared by adding

400 units of streptokinase in each ml of 0.5% bile salt broth.

6. Marrow Culture

Culture of material obtained by sternal puncture has been shown to be useful in making diagnosis of typhoid fever. According to Gerald T Keusch (1994) it will yield positive culture even in those cases where the blood culture is negative.

7. Bile Culture

Bile is obtained by means of duodenal tube and is useful in late stages particularly in detecting carriers.

SEROLOGICAL DIAGNOSIS

An attack of typhoid fever confers some immunity although second attack can occur. Marmion et al (1953) described an outbreak in which 11 men who had suffered typhoid 5 months before has second attack.

Both H(IgG and O(IgM) agglutinating antibodies develop but in some cases only one may be detected.

Widal Test

Currently this is the most popular test used for the diagnosis of typhoid fever. It measures H and O agglutinins in patient's sera.

Two methods are applied :

Slide method : a rapid method but less reliable.

Tube method : Dreyer's tube is used for 'H' agglutinin. and Relix tube for 'O' agglutinin.

According to Manson Bahr and Wilcocks (1982) usual limits of agglutinination with normal sera for S. typhi and S. paratyphi B, are 'H' 1/30, 'O' 1/50 and for S. paratyphi A is 'O' and 'H' 1/10. In our study widal titre for 'O' and 'H' antigens in the range of 1:80 dilution was taken as definite positive widal test.

Persons inoculated with typhoid vaccine (TAB) will show antibodies against S. typhi, S. paratyphi A and B but in case of infection, antibodies will be present only against infecting species.

Presence of Vi-agglutinin has considerable value in recognizing carriers of S. typhi and a titre of 1/10 is regarded as significant. But this is not useful in detecting carriers in highly endemic areas (Anderson, 1970).

Diazo Test

The earliest details about Diazo test has been described by Ehrlich in the year 1882 and the name of the test was coined after the name of scientist, as Ehrlich's Diazo test.

The Principle of test, as described by Ehrlich, is based on putrefaction of protein in intestine and the break down product of protein are excreted in urine as phenol ring compound, which give the positive test.

Adams and Maegraith (1991) found that this test is usually positive in 80-90 percent cases between fifth and fourteenth days of disease while the patient is febrile. As originally described by Ehrlich, they performed the test by adding 5 ml of diazo reagent in equal amount of fresh urine and mixing them gently. To this solution few drops of 30 percent ammonium hydroxide solution were added and mixture was shaken well. If the resultant froth gives pink or red colour, it was taken as a positive reaction.

In the year 1962, Huck step RL once again evaluated the efficacy of Diazo test in the diagnosis of typhoid fever. He recommended its utility as a diagnostic aid in typhoid fever. He found that red colouration observed on mixing the urine of typhoid patient with Diazo reagent is produced by the presence of substance containing a phenol ring formed by putrefaction of proteins in intestine. He noted that

diazo reaction is positive in typhoid fever in 80% of the cases between fifth and fourteenth day of illness, not usually as early as second day or later than the twenty first day of illness. He also found false positive reaction in 5% non typhoid patients i.e. pulmonary tuberculosis, measles and typhus.

Wilcocks and Mansen-Bahr (1982) reported this test as a useful diagnostic tool in early diagnosis of typhoid fever. He also noted the sensitivity of the test in the range of 80%.

Ananthnarayan and Pankiker (1989) have also found that diazo test is a very simple bed side test which can give an early clue to the diagnosis of typhoid. They reported that Diazo test becomes positive on 4th day of clinical illness, remains positive till acute stage lasts and becomes negative with the disappearance of the fever.

Raghuraman et al (1992) are the only workers to have evaluated this test recently in the diagnosis of typhoid fever.

They evaluated this test in a study done on 30 patients having suspicion of typhoid fever. Out of 30,

only 12 cases were blood culture positive for S. typhi. At the time of inclusion in study they all were widal negative.

A total of 149 diazo tests were done on these 30 cases during their period of hospitalization. In 12 culture positive cases, 86 Diazo tests were performed. Out of these 86 tests, 18 Diazo tests were performed when patients were not on antibiotics and rest of the 68 Diazo tests were done when patients were on antibiotics.

In the 12 culture positive cases there were 11 positive Diazo test and one negative. They observed the sensitivity of test in the range of 92% and specificity 83%. They also observed that cases with positive Diazo test remained so through out the period of illness and became negative only with clinical response. Another interesting finding was that administration of antibiotics did not interfere with the Diazo test. Raghuraman et al (1992) also reported the positive prediction value of 79% which signifies that in about 79% of occasions a positive test indicates the presence of typhoid fever.

The liklihood ratio of positive test for Diazo

test in this study was 5.48. It means that a positive test is 5.48 times as likely to come from a patient with typhoid fever as from one without the disease.

Scanning the literature has not given any further reference on this test.

AIMS AND OBJECTIVES

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Aim of study was to evaluate the role of diazo test in the diagnosis of typhoid fever.

MATERIAL AND METHODS

MATERIAL AND METHODS

The present study was carried out in the Department of Paediatrics, M.L.B. Medical College, Jhansi in active collaboration with Department of Microbiology, ober a period of 10 months from November, 1994 to September, 1995. The case material was obtained from patients attending the out patient department (OPD) and those admitted in Emergency and Paediatric ward of M.L.B. Medical College, Hospital, Jhansi.

SELECTION OF CASES

Children between the age group of 2 to 15 years attending the out patient department (OPD) as well as those admitted in the Paediatric and emergency wards of M.L.B. Medical College, Hospital, Jhansi with the clinical suspicion of typhoid fever were included in the present study.

For the clinical suspicion of typhoid fever, main criteria taken was fever with the history of 5 or more days. Other important features given due emphasis were dull and toxic appearance, high grade fever, soft and tender splenomegaly, change in bowel habit, anorexia,

and coated tongue .

HISTORY

A detailed present, and past history of illness was taken in each case. Extra attention was paid in eliciting history of pattern and type of fever as in typhoid, initially there is mild to moderate remittant fever which later on becomes high grade and continuous. History of diarrhoea during early part of illness and constipation in later part of illness was recorded. As epistaxis is not uncommon, history of bleeding per nose was also asked. Change in dietary habit, was asked as anorexia is a common feature of typhoid.

A detailed history of rashes was taken viz type of rash, site, time of appearance, and their relationship with fever. Complaint of cough was also noted as bronchitis is a common feature of typhoid fever.

A detailed past history of similar illness was taken because recurrences are common with typhoid fever. Past history of prolonged fever, anorexia, loss of weight, prolonged cough, haemoptysis was also taken.

Drugs taken by the patient were noted and every possible effort was taken to know the nature of drug recieved, especially antibiotics and the response observed by attendant was also noted.

A family history suggestive of tuberculosis was elicited. For this history of prolonged fever,prolonged cough, haemoptysis, weight loss, haemoptysis in any member of the family was asked. Leading questions were asked to elicit antitubercular therapy (ATT) given to any member of family.

Due emphasis was given to exclude tuberculosis measles, and typhus as these diseases may give false positive Diazo test.

To exclude tuberculosis, if any patient was found to be a suspicious case of tuberculosis, all relevant examination and investigations were done in order to exclude the disease. This included chest X-ray, Montoux test, sputum examination, apart from routine blood counts and ESR. Response to antibiotics given was monitored as an aid to rule out tuberculosis.

Measles was excluded with the history of fever and the sequential appearance of rash starting from face. Past history of attack of measles was procured as second attack of measles is rare. Vaccination status of cases was also noted and immunization against measles was specifically asked. Patients were carefully followed for any rash suggestive of measles. History of similar illness in other children of community was also asked.

Typhus was excluded by history and clinical presentation of patient. Patient was carefully watched for abrupt onset of high grade fever, hemorrhagic spots over body early appearance (usually on III day) of palpably enlarged spleen, early deterioration in neurological status (on 6th or 7th day), haematuria, haemoptysis, malena, gangrene of area of skin.

Socio-economic status was recorded as typhoid is common in low socio-economic strata.

EXAMINATION

A detailed general and systemic examination was done in each and every case with special reference to note the type and degree of fever. General appearance of

patient was given due emphasis as patients having typhoid fever are usually dull and toxic. Tongue was examined for coating.

Patients were carefully examined daily for rashes. If any rash was found, its character and site were carefully watched. Duration of illness on the appearance of eash was correlated with character and site of rash. Any hemorrhagic spot or gangrene of areas of skin was taken as an evidence of typhus.

Patients were searched for significant lymphadenopathy especially axillary and cervical. Enlarged multiple, non tender, matted lymph nodes without evidence of any lesion in their draining area was taken as a hint for the search of tuberculosis. These patients were further investigated by chest X-ray, Montoux test, ESR, blood counts and sputum examination to rule out tuberculosis.

Every patient was examined for pallor, icterus, bowel sound and tongue coating.

Search for splenomegaly was made and its character and type were noted carefully.

Patients who presented with the complaints of abdominal discomfort were given special attention towards complications related to gastrointestinal tract i.e. paralytic ileus, gastro-intestinal bleeding, perforation and peritonitis.

Patients having cough were examined carefully as pneumonitis and bronchitis are common features of typhoid fever.

INVESTIGATIONS

1. Blood counts and ESR

Each and every patient was investigated for Hb, TLC, DLC and ESR.

2. Urine Examination

Routine&microscopic examination of urine of each and every patient was done.

3. Blood Culture

Blood culture was done in every case with suspicion of typhoid. Precautions were taken so that samples were taken with disposible syringe and blood samples were taken prior to giving any antibiotics, in the hospital. Blood culture vials were prepared at microbiology

department of M.L.B. Medical College. For sending culture samples these vials were kept under refrigeration in the post graduate research laboratory of department. Every vial was sealed with wax to make it airtight and kept for 1 week only. After one week new vials were prepared to take precaution against contamination. Vials were taken out from refrigeration prior to blood sample collected.

4. Widal Test

Widal test was performed in every case preferably after 1 week of illness. It was repeated in every borderline case after three days of first widal test and increasing titre was given due emphasis. Test was performed by tube method for 'O' and 'H' antigens separately. Values for the ratio of 1:80 for 'O' titre were taken as positive but the test was repeated after 3 or 4 days for increasing titre.

5. Diazo Test

Diazo test was performed in each and every case under study. This was done bed side on early morning urine sample and repeated daily throughout the duration

or for eight days, which ever is earlier.

Principle

Huckstep (1962) described that when urine of the typhoid patient is mixed with Diazo reagent and is shaken, froth of the urine gives red colour. This red colour is given by phenol ring compounds produced in the intestine due to putrefaction of proteins, which are excreted in the urine.

Diazgo Reagent

Diazo reagent was prepared in research laboratory of Department of Paediatrics, M.L.B. Medical College, Jhansi. This reagent is prepared from two stock solutions namely Solution 'A' and 'B'.

Solution 'A'

It is prepared by mixing the following :

1.	Sulphanilic acid	500 mg
2.	Concentrated HCl	5 ml
3.	Distilled water	100 ml

Solution 'A' is stable solution but even then it was prepared fresh each time&the old one was discarded.

Solution 'B'

Solution 'B' was prepared by dissolving 500 mg of sodium nitrite in 100 ml of distilled water. Solution 'B' is not a stable solution. This solution was kept at approximately 4°C temperature under refrigeration. This solution was prepared fresh every three weekly.

Procedure

Forty parts of stock solution 'A' was mixed with one part of stock solution 'B'. Equal volume of this mixture and fresh early morning urine of patient were mixed in a test tube. To this, 5 drops of 30% ammonium hydroxide was added and test tube was shaken well. A positive reaction was taken as pink/red colouration of froth.

6. Other Investigations

Other relevant investigations were done in every case having possibility of some other illness.

Children showing lymphocytosis were investigated by X-ray chest, sputum examination and Montoux test to exclude tuberculosis.

O B S E R V A T I O N S

OBSERVATIONS

The present study was conducted in the department of Pediatrics, M.L.B. Medical College, Jhansi. Cases were selected from patients attending the O.P.D. and admitted in the emergency and Pediatrics ward of the hospital.

The present study comprised of 63 patients, varying from 2-15 years of age. Among them 27 patients were proved cases of typhoid fever and the remaining 36 cases were suffering from diseases other than typhoid fever. These 36 patients served as control in the study.

The following are the observations of the present study.

TABLE I : Distribution of patients according to age.

Age group (years)	Study group		Control group		Total	
	No.	%	No.	%	No.	%
2 - 5	8	29.00	18	50.00	26	41.00
6 - 10	16	59.00	14	38.00	30.	48.00
11 - 15	3	12.00	4	12.00	7	11.00
TOTAL	27		36		63	

As shown in table I, 41% patients under study were 2-5 years of age while 48% of patients were of 6-11 years age and rest 11% belonged to 11-15 years of age. Maximum number of cases were in the age group of 6-10 years of age while maximum controls were of less than five years of age.

TABLE II : Sex distribution of study and control groups cases.

Sex	Study group		Control group		Total	
	No.	%	No.	%	No.	%
Males	23	85.00	24	66.00	47	74.00
Females	4	15.00	12	34.0	16	26.00
TOTAL	27		36		63	

Table II clearly shows male dominance among study and control groups. Out of 63 patients of both the groups, 47 (74%) were males and remaining 16(26%) cases were females. Among study group 23(85%) were males while in control group 24(66%) were males.

TABLE III : Socio-economic status of study and control group cases.

Socio-economic status	Earning/ month(Rs)	Study group		Control group	
		No.	%	No.	%
Low	⟨1000	12	44.00	18	50.00
Lowermiddle	1000-2000	9	33.00	10	28.00
Middle	⟩2000	6	22.00	8	22.00
TOTAL		27		36	

Data in table III tells that majority of cases of study and control group was of low socio-economic status. Forty eight percent of total patients under study were of low socio-economic status and their distribution among study and control group was almost equal. Only 22% of patients were from middle socio-economic strata and none

of them was from high income group.

TABLE IV : History of fever at first visit.

Duration (days)	Study group No.	%	Control group No.	%
5 - 8	13	48.00	17	47.00
9 - 13	10	37.00	18	50.00
713	4	15.00	1	3.00
TOTAL	27		36	

Table IV reveals that most of the cases of the study group were having fever for 5-8 days (48%) while only 15% of cases were having fever for more than 13 days. Among the controls, 47% were having fever for 9-13 days and only 1(3%) case was having it for more than 13 days.

TABLE V(a): Other clinical features present among cases of study and control groups.

Features	Study group No.	%	Control group No.	%
Pallor	8	30.00	3	8.00
Bronchitis	12	45.00	10	28.00
Hepatomegaly	18	66.00	7	19.00
CNS involvement	22	80.00	9	25.00
Abdominal complaints.	8	30.00	5	14.00

It is evident from the table V(a) that apart from fever, splenomegaly was the most common finding and was present in 80% of cases of study group and hepato-megaly stood second and was present in 66% cases of study

group. Bronchitis was another common finding and was present in 45% of study group cases. Thirty percent of the study cases were having anaemia clinically. Abdominal complaints were present in 30% cases in the form of either diarrhoea or constipation. Only 15% patients showed the features of CNS involvement.

Among control group cases common features found were bronchitis (28%), CNS involvement (25%) and splenomegaly (19%).(Table Va).

TABLE Vb : Diagnosis of control group cases.

Diagnosis	Control group No.	%
Malaria	9	25.00
Encephalitis/Encephalopathy	8	22.00
Bronchopneumonia	6	16.00
Lobar pneumonia	4	11.00
Pyrexia of unknown origin	6	16.00
Meningitis	3	8.00

As evident from table Vb malaria was found in 25% of cases in control group. Twenty two percent cases were having encephalitis or encephalopathy. Twenty percent cases were having lower respiratory tract infections. In 16% cases causes of fever could not be found, while remaining 8% were later on proved as patients of meningitis.

Table VI clearly shows that 89% of cases were taking oral antibiotics before coming to this hospital.

In the rest of 11% cases nature of the drug received could not be traced out but they were taking some form of medicine.

TABLE VI : History of taking oral antibiotics.

	Study group	
	No.	%
Drug taken	24	89.00
Suspected(drug taken but nature of drug not known)	3	11.00
Not taken	-	-

TABLE VII : Duration of oral antibiotic administration among the cases with positive history of drug intake (n=24).

Duration (days)	No.of cases	Percentage
1 - 3	-	-
3 - 5	18	75.00
7 5	6	25.00
TOTAL	24	100.00

Table VII reveals that of the cases receiving oral antibiotics, all of them were taking it for more than 3 days and 25% of them were taking for 75 days.

Table VIII shows that 75% of the cases were on parenteral antibiotic therapy prior to coming to this hospital and only 25% cases did not receive parenteral antibiotics.

TABLE VIII : History of taking parenteral antibiotics among the study group.

Parenteral antibiotic	No.of cases	Percentage
Taken	20	75.00
Not taken	7	25.00

TABLE IX : Duration of parenteral antibiotic therapy among study group cases with positive history of injectible antibiotic administration.

Duration(days)	No.of cases	Percentage
1	7	35.00
2	8	40.00
3	4	20.00
4	1	5.00
TOTAL	20	100.00

Table IX shows that among study group cases which were receiving parenteral antibiotics, 65% cases were taking them for two or more days.

TABLE X : Total leucocyte count in study and control groups.

Total leucocyte count (cells/cumm)	Study group		Control group	
	No.	%	No.	%
⟨4000	5	19.00	1	3.00
4000 - 11000	22	81.00	35	97.00
⟩11000	-	-	-	-
TOTAL	27	100.00	36	100.00

It is evident from table X that 19% of the cases of study group were having leucopenia as against 3% of control cases. Leucocytosis was not found in any of the patient. Eighty one percent of the study group cases were having leucocyte count in normal range. Among the control cases, 97% were showing leucocyte count more than 4000 cells/cumm of blood.

TABLE XI: Results of blood culture.

Blood culture	No.of cases	Percentage
Positive for S. typhi	11	18.00
Negative for S. typhi or sterile	52	82.00
TOTAL	63	100.00

Table XI reveals that out of 63 blood cultures done, only 11 (18%) were positive for S. typhi and rest 52(82%) were sterile. All cultures positive cases later on gave widal test in positive dilution too.

TABLE XII : Results of widal test (first).

Titre for 'O' antigen	Study group		Control group	
	No.	%	No.	%
< 1 : 80	-	-	36	100.00
1 : 80	3	11.00	-	-
1 : 160	15	55.00	-	-
1 : 320	9	33.00	-	-
TOTAL	27	100.00	36	100.00

As shown in table XII that none of the case showed titre for 'O' antigen in dilution ⩽1:80 on first widal test. Hundred percent of control cases werex showing widal titre in dilution ⩽1:80. Only 3(11%) cases of study group were showing widal titre positive in dilution 1:80, while 15(55%) cases were widal positive in dilution of 1:160. However, 33% of study group cases were widal positive in dilution 1 : 320 on first widal test.

TABLE XIII : Results of second widal test in all cases of study and control groups.

Titre for 'O' antigen	Study group		Control group	
	No.	%	No.	%
⩽ 1 : 80	-	-	36	100.00
1 : 80	-	-	-	-
1 : 160	11-	41.00	-	-
1 : 320	16	59.00	-	-
TOTAL	27	100.00	36	100.00

Widal test was repeated after 3-4 days of first test in all the cases of both groups. It is evident from table XIII that on repeate widal test none of the control showed increase in widal titre, while all the 3 cases of study group who were showing first widal titre 1:80 dilution became widal positive in dilution1:160 and number of cases of study group showing widal titre in dilution 1:320 rose to 59% on repeat widal test.

As is evident from table XIV all the cases of control group were Diazo negative through out the period

of study while 25 out of 27 cases of study group (93%) were Diazo test positive.

TABLE XIV : Diazo test in cases of both the groups.

Result of Diazo test	Study group		Control group	
	No.	%	No.	%
Positive	25	93.00	-	-
Negative	2	7.00	36	100.00
TOTAL	27	100.00	36	100.00

TABLE XV : Results of total Diazo test done.

Results of Diazo Test	Study group	Control group
Positive	96	-
Negative	112	144
TOTAL	208	144

Data from table XV shows that total 352 Diazo tests were done. Out of which 144 tests were done on control cases and were negative and 208 Diazo tests were done on 27 widal positive cases of study group. Out of these 208 tests, 96 Diazo tests were positive and 112 Diazo tests were negative.

TABLE XVI : Persistence of fever after starting treatment.

Day	No.of cases	Percentage
1	27	100.00
2	27	100.00
3	20	74.00
4	10	37.00
5	2	8.00
6	-	-
7	-	-
8	-	-

Table XVI clearly shows that all of the patients were having fever for first two days after starting the treatment for typhoid fever. Fever subsided in 26% patients on 3rd day and 63% cases on day 4th and 92% cases on day 5 while all the patients were free from fever after day 5th of treatment in hospital.

TABLE XVII : Correlation of Diazo test with defervescence of fever.

Day	Diazo test +ve cases	Fever	Disparity
1	25	27	2
2	25	27	2
3	20	20	-
4	15	10	5
5	5	2	3
6	2	0	2
7	1	0	1
8	0	0	0

Table XVII shows that Diazo test has good correlation with defervescence of fever in typhoid fever. Out of 27 cases having fever on day 1 and 2, 25 were Diazo positive. After 2 day, fever started subsiding in cases taking treatment. On day 3rd, 20 cases were still having fever and were Diazo positive too. On day 4th only 10 patients were having fever but there were 15 Diazo positive cases. After day 5th, none of the case was having fever while 2 and 1 cases were Diazo positive on day 6th and 7th respectively. On day 8th no case was having either fever or positive Diazo test.

TABLE XVIII : Disappearance of positive Diazo test in correlation with defervescence of fever.

Parameters	No.of cases	Percentage
Diazo disappeared before defervescence of fever	3	12.00
Both event occurring on same day	11	44.00
Diazo disappearing after defervescence of fever	11	44.00
TOTAL	25	100.00

Table XVIII shows that in 12% of cases Diazo test became negative before defervescence of fever. In all these cases this event occurred just one day before. In 44% of Diazo positive cases, test became negative on the very same day of defervescence of fever. In remaining 44% of Diazo positive cases, it became negative after one or two days of defervescence of fever.

D I S C U S S I O N

DISCUSSION

Typhoid fever is diagnosed traditionally by blood culture and widal test though other tests i.e. urine culture, stool culture, marrow culture etc, can also diagnose it. Out of these above mentioned tests, widal test is the only simple test and is most widely used, but even this is useful only after 1 weeks of illness. The only other test, which can diagnose typhoid fever earlier than widal test is blood culture which is not a simple test and it has its own limitations.

Since typhoid fever is a common cause of acute febrile illness, there is need of test which is simple and can diagnose typhoid fever early.

Diazo test has given some hope as a simple bed side test which van diagnose typhid fever even in first week ofillness.

Preasent study was aimed to evaluate the Diazo test in the diagnosis of typhoid fever.

The study comprised of 63 patients. Out of these, 27 patients later on proved to be cases of typhoid fever because of positive widal test, while rest of the 36 patients served as controls in which widal test was found to be negative.

It is evident from table I that maximum number of patients of typhoid fever belonged to age group 6-10

years)59%). This data just gives a clue that in pediatric age group, typhoid fever is commoner in school age children. Only 12% of cases of study group were more than 11 years old. Youngest patient in this study was 2.5 years old male child, while the oldest patient was a 13 years old female child.

Kaul et al (1990) in their study of 48 cases found that 45.8% of cases were in age group of 0-5 years while in study done by Pandey et al (1990) only 32.3% cases were in age group less than five years.

Kapoor et al (1990) in their study on typhoid fever in children, found that 53.3% of cases came from age group of ⩽5 years.

Mishra et al (1991) also reported that 38% of these patients came from age group 2-5 years, while rest of the cases were in the age group 5-19 years.

Sharma and Gathwala (1992) in their study on 69 children found that 21.5% patients were in age group less than five years, 32.3% were between 6 to 10 years and 46.3% were between 11 to 14 years.

As far as sex is concerned table II shows that 75% of the total patients under study were males. Among typhoid fever cases 85% were males,while 66% of controls were males. The male preponderance could be a reflection of the traditional Indian family taking more interest in the male sib in all spheres including medical attention.

In the study conducted on typhoid fever by Biswal et al (1993) , 70% of the patients were of more than 5 years of age, while rest of them were of less than 5 years of age.

Forty four percent of the cases in our study were from low socio-economic background (Table III)., and 33% were from lower middle strata. Together they constituted 77% of the cases. Only 23% of cases were from middle socio-economic group. None of the case, however belonged to higher income strata group. This finding was expected as typhoid fever is spread by faeco oral route and failure in personal hygiene and particularly in public health precautions commonly results in appearance of typhoid fever.

In this study main criteria for selection of cases was history of fever for more than 5 days. It is evident from the table IV that 48% cases were having fever from 5-8 days and 52% cases were having fever for more than 8 days. Average duration of fever at the time of first visit to this hospital was 9.25 days per patients. These results were comparable to other studies done. Kaul et al (1990) in their study reported average duration of fever from 9.23 to 10.18 days. Sharma and Gothwala(1993) in their study on multi drug resistant typhoid fever found that average duration of fever was from 13.1±9 days to 15.8±8.2 days.

As shown in table V apart from fever, other common clinical features were splenomegaly (80% cases), hepatomegaly (66%, and bronchitis in 41% cases. Pallor was present in 30% cases,while CNS involvement was found in 15% cases. Various workers in the past have commented upon the clinical profile of cases of typhoid fever.

Marmion (1952) found splenomegaly in upto 70% of cases during the period of illness.

Mishra et al (1991) in their study on multi-drug resistant typhoid fever reported that splenomegaly was detected in 60% of cases, while hepatomegaly was present in 90% cases. They also noted bronchitis in 35% of their cases.

Sharma and Gathwala (1992) reported splenomegaly in 36% cases, while hepatomegaly in 25.8% of cases. Similar results were obtained by Kohl et al (1990).

Biswal et al (1993) reported splenomegaly in 65% of cases and hepatomegaly in 62% of cases of typhoid fever. Features of bronchitis were present in 21% of cases in their study.

The reason for high percentage of splenomegaly in our patients might be endemicity of malaria in Bundelkhand region.

It is evident from table VI that out of 27 proven cases of typhoid fever, 89% of cases had taken oral antibiotics prior to admission in our hospital. However, 3 cases were giving the history of drug intake

but the nature of antibiotic could not be confirmed from the history or from the other records. Out of the cases taking antibiotics orally, all of them were doing so for more than 3 days and 25% of them were taking oral antibiotics for more than 5 days (Table VII). On enquiring about the nature of antibiotics, it was found that most of patients were either taking Amoxycillin or Ampicillin or Ampicillin and Cloxacillin combination or chloramphenicol, while some patients were even on quinolones.

As shown in table VIII, 20 out of 27 cases (74%) were also taking parenteral antibiotics and table IX shows that 75% of them were taking these antibiotics for more than one day.

It is evident from table X that leucopenia was present in 19% of the cases, while only 3% of control group (1 patient) were leucopenic. Majority of cases were having leucocyte count in normal range of 4000-11000 cells/cumm blood.

Mishra et al (1991) in their study found leucopenia in 12% of cases, while 72% of cases were having leucocyte count in normal range.

Keusch GT (1994) reported leucopenia in 25% cases of typhoid fever and majority was having normal leucocyte count.

Leucocyte count obtained in our study was comparable with other studies and no significant difference was found.

It is evident from table XI that out of total 63 cases only 11 cases showed a positive blood culture for S. typhi and all of them were widal positive too.

Keusch GT (1994) had reported that 90% of cases of typhoid fever give positive blood culture for S. typhi in first week of illness and the positivity falls to 50% after 2nd week of illness. He further reported that a positive blood culture is usually obtained when patients are not on antibiotic and appropriate culture techniques are employed.

Ananthnarayan and Panikar (1989) also found that blood culture is positive for S. typhi in 90% cases of typhoid fever in first week of illness and in 60% cases after 2nd week of illness. These values are obtained only if blood culture is done prior to administration of antibiotic and proper culture techniques were employed.

The low blood culture positivity of only 40% observed by us can be attributed to the fact that all the cases had taken either oral or parenteral or both the forms of antibiotics, atleast for 3 days or more prior to hospitalization and blood culture estimation.

As widal test was performed in each and every patients and it was the main criteria in deciding cases and controls, all widal negative patients served as controls.

According to Manson Bahr and Wilcocks (1982) upper limit of negative widal test is 1 : 30 dilution for 'H'

antigen and 1 : 50 dilution for 'O' antigen. In our study widal test in dilution of 1 : 80 for 'O' and 'H' antigen was taken as positive reaction.

It is evident from table XII that all the cases were widal positive in the dilution 1:80 or more on further categorising them, it was seen that 11% cases were widal positive in dilution 1 : 80 on first instance, while 55% cases were positive in dilution 1 : 160. Thirty three percent cases however, had a titre of 1 : 320 for 'O' antigen on first widal test.

When widal test was repeated after 3-4 days, it was seen that the 3 cases, which had widal positivity in dilution 1 : 80 recorded a higher titre and became positive in dilution 1 : 160. Out of 15 cases which had widal positivity in dilution 1 :160, only seven cases showed an increasing titre of 1 : 320 on serial widal test.

Diazo test was performed in each and every patient every day from the first day of contact. Even if any patient was found Diazo negative on very first day, we continued to repeat the test for atleast four days and stopped doing so if patient remained Diazo negative for these four days.

If on very first day, Diazo was found positive in any patient then test was done daily for eight days or till the date of discharge of patient, which ever is earlier.

Total 352 Diazo tests were done on 63 patients. Out of these, 208 Diazo tests were done on the 27 widal positive cases and rest 144 tests were done on the 36 widal negative (Table XV) cases which served as controls in the present study.

Diazo test was found negative in all the 36 controls from the very first day of study and remained so throughout the course of their illness (Table XIV) (specificity - 100%). Such a high specificity was expected as measles, tuberculosis and typhus cases were excluded from the study as they can give false positive results.

Out of 216 Diazo tests done on 27 widal positive cases, 94 tests were positive. All of these 94 tests were done during the early part of illness, while rest 122 negative Diazo tests were done after defervescence of fever (Table XVI). As evident from table XIV, two widal positive cases remained Diazo negative throughout the course of their illness, while rest 25 cases were Diazo positive (Sensitivity = 85.2%).

An interesting finding from table XVII, is that 25 cases were Diazo positive from the very first day of study, and at that time fever was also present in all the cases. On third day of study only 20 patients were having fever and same patients were Diazo positive too. On day 4th, only 10 cases were having fever, while 15 cases were still Diazo positive. Similarly on day 5th, only 2 cases were having fver against 5 Diazo positive cases. After

day five onwards, no patient was having fever, while only 2 cases were Diazo positive on day sixth and one on day 7th (Table XVII). These findings clearly indicate that Diazo test has good correlation with clinical illness. As soon as fever started subsiding after starting treatment, cases also started becoming Diazo test negative.

It is evident from table XVIII that Diazo test has good correlation with defervescence for fever and thus with clinical improvement of patient of typhoid fever.

Diazo test was positive in 92.6% of cases of typhoid fever when disease was in active state. As shown in table XVIII, in 44% of cases, Diazo test became negative on the same day of defervescence of fever, while in another 44% cases Diazo test remained positive for one or two days after fever subsided. Only in 12% of cases the Diazo test became negative a day prior to defervescence of fever.

Similar inferences were drawn by Raghuraman et al (1992) in their study conducted on 30 cases having suspicion of typhoid fever. Out of these 30 cases 12 cases were proven cases of typhoid fever on the ground of positive blood culture for S. typhi while rest of the 18 cases were taken as controls. They observed that among 12 cases, 11 were Diazo positive too and only one case was Diazo negative. Diazo test in eleven cases remained positive throughout the period of illness and became

negative only with clinical response. They calculated the sensitivity and specificity of this test and reported a sensitivity of 92%, while the specificity was 83%.

A very interesting observation of our study as regards the Diazo test, a simple bed side technique for early diagnosis of typhoid fever was a higher specificity of this test than that observed by Raghuraman et al(1992).

A higher specificity in our study was possible as we excluded diseases like measles, tuberculosis and typhus which give a false positive reaction. Besides being a highly specific test, the diazo test is also not adversely affected by the administration of antibiotics, which is the greatest drawback with the positivity of blood culture, the gold standard in the diagnosis of typhoid fever.

Thus in a nutshell our observation reveal that Diazo test besides being a simple, early bed side test is also highly specific and should be considered as a diagnostic aid in the early diagnosis of typhoid fever.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The present study was conducted in Department of Pediatrics, M.L.B. Medical College, Jhansi for a period of 10 months from Nov., 1994 to Sept., 1995. The cases included in this study comprised of children of 2-15 years of age presenting with the suspicion of typhoid fever.

Altogether 63 children presenting with the history of fever for more than 5 days with the suspicion of typhoid fever were studied. The aim of study was to evaluate the Diazo test in the diagnosis of typhoid fever.

A detailed history regarding the character and type of fever, anorexia and change in bowel habit was taken. Detailed drug history was also noted.

Each and every patient was carefully examined for toxic look, coated tongue, splenomegaly, hepatomegaly, rashes and pallor.

The diagnostic procedures employed for detecting typhoid fever cases were blood counts, haemoglobin, blood culture and widal test.

All the cases of tuberculosis, measles, and typhus were excluded from the study. For this, a detailed history and clinical examination was done. If any case was found having suspicion of above mentioned disease, relevant investigations were done to confirm the diagnosis.

In all the cases under study, Diazo test was done on early morning urine sample, every day for eight days if any patient showed positive Diazo test, otherwise if patient did not show positive Diazo test for first four days, it was stopped and patient was regarded as Diazo negative.

The results obtained were summarised as follows :

1. Only 20% of the cases were of age less than 5 years of age, while most of the cases (59%) were in the age group of 6-10 years.

2. There was a definite male preponderance in the study and control groups. Among the cases, 85% were males, while among the controls 66% were males.

3. Most of the cases were from lower socio-economic status viz. 77%, while only 22% cases belonged to middle socio-economic strata. None of the case was from high income group.

4. All of the cases were having fever for more than 5days as it was the main criteria for selection of cases and controls. Among cases 48% were having fever for 5-8 days, 37% were having fever for 9-13 days and 15% were having fever for more than 14 days.

5. Apart from fever the other common clinical features were splenomegaly in 80% cases, hepatomegaly in 66%, and bronchitis in 41% of the cases. Pallor and abdominal complaints were present in 30% cases, while involvement had occurred in 15% of cases.

6. Among control group cases, which comparised of 36 patients there were 9 cases of malaria, 8 cases of encephalitis/encephalopathy, 6 cases were of bronchitis and pyrexia of unknown origin each and 3 cases of meningitis.

7. Twenty four out of 27 cases were taking oral antibiotics and rest of the 3 cases were also taking drugs but nature of drug could not be traced out. Among cases taking oral antibiotics, all of them were doing so far more than 3 days, while 25% of them were taking oral antibiotics for more than 5 days.

8. Twenty out of 27 cases were taking parenteral antibiotics too and 65% of them were taking them for two or more days.

9. On investigating them for leucocyte count, 19% cases showed leucopenia, while rest of the 81% were having leucocyte count in normal range. Non of the cases showed leucocytosis.

10. Among the 27 widal positive cases, 11 were blood culture positive for S. typhi.

11. On first widal test, done after 1 week of clinical illness, 27 out of 63 patients under study showed positive results, while rest of the 36 patients showed negative widal test and served as control in the study. Among the widal positive cases, 11% cases were positive in dilution of 1 : 80 for 'O' antigen, 55% were positive for dilution 1 : 160 and rest 33% were positive for 1 : 320 dilution for 'O' antigen.

12. On repeat widal test, done 3-4 days after the first one, all the 3 cases showing widal titre of 1 : 80 showed an increased titre of 1 : 160, while 7 cases of 1 : 160 dilution in first widal became positive in dilution 1 : 320. None of the control group patient showed any increase in widal titre value.

13. Total 352 Diazo tests were done on 63 patients under study. Out of 352, 208 Diazo tests were done on cases and 144 were done on controls.

14. All the Diazo tests done on controls were negative, while out of 208 Diazo tests done on 27 cases, 86 were positive, which were done in early part of

illness, while rest of the negative 112 Diazo tests on the cases were done after defervescence of fever.

15. All the control group patients were Diazo negative from the very first day of study and remained so throughout their course of disease, while 25 out of 27 typhoid cases were Diazo positive during the active stage of illness.

16. There was a strong correlation between Diazo test results and defervescence of fever. It was found that out of 27 cases having fever on day 1, and 2, 25 were Diazo test positive. On day 3, only 20 patients were having fever and Diazo test positive. On the day 4, fever was present in only 10 cases, while 15 cases were showing positive Diazo test. On day 5, only 2 patients were having fever, while 5 cases were Diazo positive. On day 6 of study and onwards no patient was having fever, while only 2 and 1 patient were Diazo test positive on day 6 and 7 respectively.

17. It was noted that in 72% cases Diazo test became negative one day prior to fever, while in 44% cases both event occurred on the very same day. In rest of the 44% cases Diazo test became negative after one or two days of defervescence of fever.

18. In our study we observed the sensitivity of Diazo test 85.2%, while specificity was 100%. A higher specificity observed by us was possible as we excluded diseases like measles, tuberculosis and typhus which give a false positive reaction.

CONCLUSION

This study amply justified that Diazo test is a useful diagnostic test in the early diagnosis of typhoid fever. More importantly prior administration of antibiotics does not interfere with the test and it becomes negative only after clinical response. This simple and quick bed side test has also a high degree of specificity and sensitivity.

B I B L I O G R A P H Y

BIBLIOGRAPHY

1. Adams and Maegraith. Clinical tropical diseases, 4th edition, Blackwell Scientific Publications, p. 455-463.

2. Ananthnatayan R and Panikar J. Enterobacteriaceae Salmonella. Text book of microbiology, III edn. Orient Long man, 1989; p. 277-292.

3. Anderson ES and Smith HP. B.M.J., 1972; 3:329-31.

4. Arneil and Forfar's Text book of paediatrics. Campbell, McIntosh, 4th edn, Churchill Livingstone, p. 1380-1382.

5. Biswal et al. Indian Peediat, 1994; 31 : 813-19.

6. Dutt PR. Rural health services in India, Central Bureau of Health Education, New Delhi.

7. Keush GT. Salmonellosis, Harrison's Principles of Internal Medicine, 13th edn, McGraw Hill, 1994.

8. Kumar R, Mishra PK, Kumar S, Testing test. Indian Pediatr, 1992; 29 : 667-672.

9. Kaul et al. Indian Pediatr, 1991; 28 : 357-361.

10. Mishra et al. Indian Pediatr.1991; 28 : 1171-74.

11. Manson Bahr. Apted FIC salmonellosis in Manson's tropical diseases, 18th edn. London Bailliere tindall, 1982; 380-390.

12. Nelson Text book of Paediatrics, W.B. Saunders, 14th edn, p. 731-734.

13. Pandey et al. Indian Pediatr, 1990; 27 : 153-56.

14. Patnaik KC and Kapoor PN. Indian J Med Res, 1967; 55 : 228.

15. Park JE and Park K. Typhoid fever. Text book of SPM, 13th edn. Banarsidas Bhanot, Jabalpur, 1992.

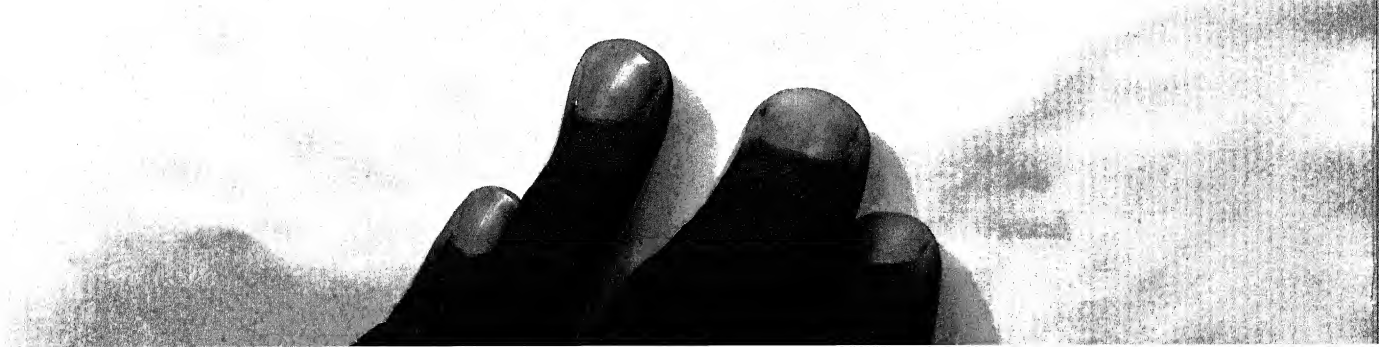
16. Raghuraman et al. Diazo test in typhoid. Indian Pediatr, 1994; 34 : 210-214.

17. Ramesh Kr et al. Am Nat Acad Med Sci (India), 1988; 24 (4) : 255-257.

18. Sharma et al. Indian Pediatr, 1993; 30 : 47-50.

19. WHO. World health statistics, 1973; 26 : 2-10.

A P P E N D I X



WORKING PROFORMA

EVALUATION OF DIAZO TEST IN THE DIAGNOSIS OF TYPHOID FEVER

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Case No.

Patient's Name MRD No.

Age/Sex D.O.A.

Socio-economic status: Ward/Bed:

History of present illness

- Duration of fever.
- Type of fever
- Character of fever
- Other complaints

Past History

- History of chronic fever
- History of fever with rashes
- Weight loss
- Chronic cough
- Night sweats
- Any others

Family History (Esp. Tuberculosis)

Drug History (Esp. Antibiotics)

General Examination

General appearance	Pallor
Level of consciousness	Icterus
Temperature	Lymphnodes
Pulse rate & B.P.	Bowel sound
Skin(for rashes, purpuric spot)	Tongue

Systemic Examination

C.V.S.

Resp. System

C.N.S.

Level of consciousness

Higher centres

Cranial nerve

Sensory Exam.

Motor Exam.

Abdomen

Bowel sound

Shape

Liver

Spleen

Any other findings

Investigations

Hb gm% TLC : cells/cumm.

DLC : P %, L %, E %, B %, M %.

Urine : Routine :

Microscopic :

Blood culture

Widal test : 1st Repeat

Diazo test on day - 1, 2, 3, 4, 5, 6, 7 and 8
